2 ALCOHOLS BY HEADSPACE GC

2.1 Summary

2.1.1 An aliquot of specimen is diluted semi-automatically with an internal standard (IS) solution (n-propanol) into a glass vial, sealed, and placed in a heated automatic sampler. The concentration of ethanol or other volatile in a dilute aqueous biological sample is directly proportional to its concentration in the gas phase or headspace. A portion of the resultant headspace vapor above the liquid is automatically injected into a gas chromatograph (GC) equipped with a flame ionization detector (FID). Alcohol (ethanol), methanol, acetone and isopropanol are identified by retention time and their concentrations are calculated by comparison to similarly treated aqueous calibrators by using peak heights or areas.

2.2 Specimen Requirements

2.2.1 50 µL of fluid(s) is diluted with 450 µL of internal standard for analysis.

2.2.2 Analyze two different biological samples for postmortem cases if possible (e.g. blood and vitreous).

2.2.3 Tissue homogenates. It may be advantageous to prepare larger volume tissue homogenates so that the additional volume may be used in other testing.

2.2.3.1 Weigh approximately 2 g of tissue on a balance. Record weight.

2.2.3.2 Place tissue in homogenizer tube and add 6 mL dH_2O. Homogenize.

2.2.4 50 µL of tissue homogenate is diluted with 450 µL of internal standard for analysis. Multiply the final result by the dilution factor (x4).

2.2.5 Tissue. Weigh approximately 0.5 g of tissue and transfer to a headspace vial. Record the weight of tissue. Pipet 4.5 mL internal standard into the headspace vial.

2.3 Reagents And Standards

2.3.1 Reference standard ethanol solutions (e.g. National Institute of Standards and Technology (NIST) or NIST traceable)

2.3.2 Reference standard ethanol, methanol, acetone, isopropanol (e.g. Cerilliant Multicomponent Alcohol Mix (A-057) Ampoules contain 500 µg/mL (0.05%) of acetone, methanol, ethanol and isopropanol)

2.3.3 Absolute ethanol (U.S. Indust. Chem. Co., New York, NY, or equivalent)

2.3.4 Methanol, isopropanol, acetone, and n-propanol

2.4 Calibrators, Controls And Internal Standards

2.4.1 Ethanol and volatile working calibrator solutions. The calibrators may be prepared as ethanol only or ethanol plus the other volatiles.

2.4.2 0.50% Ethanol and 0.25% mixed volatile calibrator. Pipet 634 µL absolute ethanol and 317 µL acetone, isopropanol and methanol into a 100 mL volumetric flask containing dH_2O. QS to volume with dH_2O. Prepare a one liter volume if using dilution preparation for calibrator preparation.

2.4.3 0.20% Ethanol and 0.10% mixed volatile calibrator. Pipet 253 µL absolute ethanol and 127 µL acetone, isopropanol and methanol into a 100 mL volumetric flask containing dH_2O and QS to volume with dH_2O. Alternately, add 40 mL of 0.50% calibrator to a 100 mL volumetric and QS to volume with dH_2O.

2.4.4 0.10% Ethanol and 0.05% mixed volatile calibrator. Pipet 126.7 µL absolute ethanol and 63 µL into acetone, isopropanol and methanol into a 100 mL volumetric flask containing dH_2O and QS to volume with dH_2O. Alternately, add 20 mL of 0.50% calibrator to a 100 mL volumetric and QS to volume with dH_2O.

2.4.5 0.05% Ethanol and 0.025% mixed volatile calibrator. Pipet 63.4 µL absolute ethanol and 32 µL acetone, isopropanol and methanol into a 100 mL volumetric flask containing dH_2O and QS to volume with dH_2O. Alternately, add 10 mL of 0.50% calibrator to a 100 mL volumetric and QS to volume with dH_2O.

2.4.6 0.01% Ethanol and 0.005% mixed volatile calibrator. Pipet 12.7 µL absolute ethanol and 6 µL acetone, isopropanol and methanol into a 100 mL volumetric flask containing dH_2O and QS to volume with dH_2O. Alternately, add 2 mL of 0.50% calibrator to a 100 mL volumetric and QS to volume with dH_2O.

2.4.7 Internal standard (IS) preparation: n-propanol. Other internal standards such as methyl ethyl ketone or t-butanol may be used if n-propanol contamination is suspected. n-Propanol can be a putrefactive product.

2.4.7.1 Stock Solution 0.3% (v/v) n-propanol. Pipet 3.0 mL n-propanol into a 1.0 L volumetric flask and QS to volume with dH_2O.

2.4.7.2 Working Stock Solution 0.03% (v/v) n-propanol. Pipet 100 mL of the stock IS solution into a 1.0 L volumetric flask and QS to volume with dH_2O.

2.4.8 Controls.

2.4.8.3 Cerilliant ethanol controls: 0.05% (E-029), 0.10% (E-031), 0.20% (E-032) and 0.30% (E-033), 0.08% (E-030)

2.4.8.4 Negative control (dH_2O)

2.4.8.5 Mixed 0.10% volatile control. Pipet 126.7 µL absolute ethanol, acetone, isopropanol and methanol into a 100 mL volumetric flask containing dH_2O and QS to volume with dH_2O.

2.5 Apparatus

2.5.1 Gas chromatograph with data system, flame ionization detector and a head space auto sampler

2.5.2 Column. Restek Rtx-BAC 1 or BAC 2 capillary column

2.5.3 Glass 20 mL (23 x 75 mm) headspace vials with Teflon septa and aluminum seals

2.5.4 Vial seal crimper

2.5.5 Test tubes or sample cups

2.6 Procedure Case samples are prepared and analyzed in duplicate. Calibrators and controls are analyzed singly.

2.6.1 Pour approximately 0.2 mL of calibrator, control, blood or other specimen into a clean test tube or sample cup (this initial step enables visualization of any clots and prevents possible contamination of the original sample by the internal standard solution). NOTE. If analyzing weighed tissue specimens (see 2.2.4), place the tissue in the headspace vial and add 4.5 mL internal standard solution. Continue with step 2.6.6.

2.6.2 Place the dilutor delivery tip into the specimen, making sure its tip is below the surface of the sample. Activate the dilutor. At this point, the dilutor draws 0.05 mL of sample into its delivery tube.

2.6.3 Withdraw the tip and wipe it with a Kimwipe/tissue paper.

2.6.4 Direct the delivery tip into the appropriately labeled headspace vial and activate the dilutor. The dilutor will dispense the specimen and 0.450 mL of IS solution into the vial.

2.6.5 Flush the dilutor tube as necessary by activating the dilutor one or more times or rinsing with dH_2O, depending on the viscosity or other nature of the specimen. Dispense washings into a waste beaker.

2.6.6 Stopper the headspace vial with the Teflon seal. Vortex or manually shake the vial for several seconds, and place in the sample rack.

2.6.7 Repeat steps 2.6.1 – 2.6.6 for all calibrators, controls and specimens.

2.6.8 Seal all headspace vials by crimping the aluminum rings over the Teflon seals.

2.6.9 Load headspace vials in the headspace auto sampler.

2.7 Headspace Analysis

2.7.1 Gas Chromatograph Operational Parameters. The following conditions are recommended starting parameters. Instrument conditions may be adjusted to permit improved performance.

- Oven 40ºC Isothermal
- Injector 200ºC
- Detector (FID) 250º
  - Hydrogen flow 35 mL/min
  - Air flow 450 mL/min
  - Make-up flow 22.6 ml/min
  - Make-up gas helium
- Inlet
  - Split
  - Split ratio 0.5:1
  - Split flow 3.2 mL/min
  - Total flow 12.8 mL/min
  - Pressure 18 psi constant pressure mode

2.7.2 Headspace Sampler Operational Parameters. The following conditions are recommended starting parameters. Autosampler parameters may be adjusted to permit improved performance.

- Sample Oven 70ºC
- Sample Valve 85ºC
- Transfer Line 95ºC
- GC Cycle 4.0 min
- Sample Equilibration 3.0 min
- Vial Pressurization 0.91 min
- Loop Fill 0.20 min
- Loop Equilibration 0.05 min
- Sample Inject 1.00 min
- Oven Stabilization 1.0 min
- Agitation Low
- Extractions 1
- Puncture Mode Single

2.7.3 Calibration check (Pre-run). The method calibration is checked at least once prior to each day's batch sample analysis. Analyze the 5 calibrators, negative control, 0.08% positive control and the 0.10% mixed volatile control on the pre-run batch. If the calibrators and controls satisfy quality control criteria, the method may be used as is or recalibrated. If the calibrators and/or controls do not satisfy quality control criteria, recalibrate the method.

2.7.3.1 A case sample(s) may be analyzed on a successful pre-run provided it is bracketed by the positive controls.

2.7.4 Batch sample analysis. Headspace alcohol analysis is performed as a batch analysis. Analyze one control after every 10 injections. The ethanol controls are: 0.05%, 0.10%, 0.20%, and 0.30% by weight by volume.

2.7.5 Start the GC by selecting "Run Sequence" under the Run Control menu. Select "Start" on the headspace display monitor to begin the analysis.

2.7.6 Vial Verification. After the completion of a volatile batch, the identity of each vial in the headspace sampler is verified with the sequence table and the alcohol worksheet. Vial verification is documented by initials and date.

2.8 Calculation

2.8.1 Volatiles are identified based on relative retention times compared to calibrators. Identification is performed by instrument software. Retention times for both analyte and internal standard must be within ± 2% of the retention time obtained from the calibrators.

2.8.2 Concentration is calculated automatically by the software based on linear regression of the 5 point calibration curve based on peak area or peak height measurement.

2.8.3 Tissue concentration is calculated as follows:

$$\text{Chromatogram concentration} \times \frac{0.5\ \text{g}}{\text{weighed amount}} = \text{volatile tissue concentration \% (w/w)}$$

2.9 Quality Control And Reporting

2.9.1 Calibration check (Pre-Run). Acceptable tolerance for ethanol calibrators is ± 6% of the target concentration or 0.004% w/v, whichever is greater. Sample batches may not be analyzed prior to an acceptable pre-run.

2.9.2 Negative control. The negative dH_2O control is used to check for carryover. An acceptable negative control may not contain ethanol. If unacceptable, prepare a fresh negative control. Reinject the 0.50% calibrator followed by the new negative control. If ethanol is still present, perform instrument maintenance to correct the problem.

2.9.3 Positive controls. Acceptable tolerance for ethanol controls is ± 6% of the target concentration or 0.004% w/v, whichever is greater. Acceptable tolerance for methanol, acetone and isopropanol in the 0.10% mixed volatile control is ± 20% of the target concentration. If a control is unacceptable, positive samples bracketed between unacceptable control(s) are repeated on another batch. Negative samples may be reported. Document actions and exceptions.

2.9.4 Correlation of determination (r^2). The r^2 value for the ethanol linear regression curve must be 0.995 or greater.

2.9.5 Duplicate tolerance. Calculate the average of the duplicates. Calculate 5% of the average and a ± 5% range. Replicates must be within the ± 5% range or within ± 0.004% (w/v), whichever is greater. Reanalyze the sample if it is outside of tolerance. An exception for duplicate tolerance of up to 20% may be made on tissue homogenates if the exception is noted in the case notes by the toxicologist.

2.9.6 Calibrator and control certification. The NIST reference standard should be analyzed with the pre-run to certify ethanol when new calibrators are prepared or a new control lot is used. The Cerilliant Multicomponent Alcohol Mix (A-057) should be analyzed to certify methanol, acetone and isopropanol in calibrators and/or controls.

2.9.6.1 The new ethanol calibrator or control must be within ± 6% of its target concentration or 0.004% w/v, whichever is greater. The NIST reference standard on the run must be within ± 3.33% of the NIST nominal target concentration.

2.9.6.2 The new mixed acetone, isopropanol, methanol calibrator or control must be within ± 10% of its target concentration or 0.004% w/v, whichever is greater. The Cerilliant Multicomponent Alcohol Mix standard on the run must be within ± 6% of the Cerrilliant target concentration or 0.004% w/v, whichever is greater.

2.9.7 Reporting.

2.9.7.1 Report the volatile concentration of the lower replicate in % by weight by volume truncated to two decimal places.

2.9.7.2 Report volatile concentrations less than 0.01% as “Not Detected.” The limit of detection and quantitation for this procedure is defined to be 0.01%

2.9.7.3 Report methanol or isopropanol concentrations $\geq$ 0.01% in embalmed cases as “Present.”

2.10 References

2.10.8 L. C. Nickolls, "A Modified Cavett Method for the Determination of Alcohol in Body Fluids," Nov. 1960, Analyst, Vol. 85, pp 840-942.

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2.10.10 K. M. Dubowski, "Manual for Analysis of Ethanol in Biological Liquids," Department of Transportation Report No. DOT TSC NHTSA-76-4, Jan 1977.

2.10.11 G. Machata, "Determination of Alcohol in Blood by Gas Chromatographic Head Space Analysis," Clin Chem. Newsletter, 4(1972), 29.

2.10.12 B.L. Levine, Principles of Forensic Toxicology, American Association for Clinical Chemistry, Inc., 1999, p. 180.

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